

SELF-INCOMPATIBILITY IN SPECIES OF *LYCOPERSICON* SECT. *ERIOBERSICON* AND HYBRIDS WITH *L. ESCULENTUM*¹

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INTRODUCTION

THE SUBGENUS *Eriopersicon* C. H. Mull. of *Lycopersicon* Mill. is made up of eight green-fruited species and varieties native to Ecuador, Peru, Bolivia, Chile, and the Galapagos Islands (Muller, 1940). Interest in this group has been aroused in recent years by the discovery that some of its members possess resistance to a number of tomato diseases (Alexander *et al.*, 1942; Virgin, 1940; Wright and Lincoln, 1940; and others).

Hybrids between the red-fruited species (subgenus *Eulycopersicon* C. H. Mull.) and species of *Eriopersicon*, in addition to the difficulty of their production, have been found to be rather sterile in spite of the fact that the behavior of meiotic chromosomes was normal in the first hybrids studied (Lesley and Lesley, 1943). MacArthur and Chiasson subsequently (1947) attributed the sterility of the F₁ of *L. esculentum* Mill. with *L. peruvianum* (L) Mill. to "cryptic structural hybridity." In a new hybrid of the same species Margaret M. Lesley (1950) found evidence of an inversion and thereby accounted partly for its sterility. Bohn (1948) was able to produce sesquidiploid hybrids by crossing 4N *L. esculentum* as the pistillate parent with 2N *L. peruvianum*. While this method made crossing much easier, and promises interesting results if the hybrids yield progeny, it shed no light on the problem of sterility of the diploid hybrid. An indication of the nature of this sterility was reported by Lamm (1950), who found *L. peruvianum* to be self-incompatible with inheritance conforming to the *Nicotiana* scheme. His study was of a preliminary nature, however, and did not include inter-specific hybrids.

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The present study was stimulated by the observation that isolated plants of the green-fruited species set fruits very poorly or not at all, while plants in groups were fruitful. In preliminary tests, branches of several plants of *L. peruvianum* were caged and their flowers were self- and cross-pollinated. While fruits were set by most of the cross-pollinated flowers, none was set after self-pollination (see table 2). Flowers that had not been hand-pollinated within the cages did not set fruit. All available species of *Eriopersicon* were subsequently tested for compatibility relations.

The literature on self-incompatibility need not be reviewed here since this work has been thoroughly done by Sears (1937), Stout (1938), and Lewis (1949).

MATERIALS AND METHODS

All plant material investigated was obtained directly or indirectly from two collections: 1) Blood and Tremelling 1937-38, in Peru, Ecuador, and Chile; and 2) Rick, 1948-49, in Peru and Ecuador. The species studied were, according to Muller's (1940) concepts: *Lycopersicon peruvianum* (L) Mill., *L. peruvianum* var. *dentatum* Dun., *L. peruvianum* var. *humifusum* C. H. Mull., *L. hirsutum* Humb. and Bonpl., *L. hirsutum* f. *glabratum* C. H. Mull., and *L. gladulosum* C. H. Mull. Table 1 gives pertinent data on all collections.

Three methods of isolation were used to prevent pollination by insects: 1) In the field, branches of plants were enclosed in wire-frame cages covered with two layers of cheesecloth; 2) inside a screenhouse—covered with a fine mesh plastic screen—which had a spring-closed door at each end of an entry-way, many plants were grown; and 3) inside a greenhouse, where efficient isolation also was obtained. The best isolation and growing conditions of the whole year were found in the greenhouse at Davis during January, February, March, and April.

POLLINATION TECHNIQUES

Two methods of pollination were used. One method was used for small numbers of flowers. These flowers were most conveniently self- or cross-pollinated with a spear-point dissecting needle whose point was somewhat rounded to slit the anthers apart and scrape pollen out of the sac. This pollen was then applied to the stigma of the desired flower. In selfing, frequently the pollen was obtained from one flower and applied to several others on the same plant, although no general rule to this effect was followed.

The other method was used where a large number of crosses were to be made with pollen from a single parent. Pollen was collected from all the flowers on one or several inflorescences with a mechanical collector consisting of a flashlight, a doorbell buzzer with a loop of wire soldered to the buzzer arm, and a glass collecting cup. This was a modification of the device described by Cottrell-Dormer (1945). The pollen thus collected was good for several days if left in the greenhouse, or longer if stored at low humidity and low temperature, according to previous tests made by McGuire (1952). Pollen was transferred with a matchstick which had been blackened with India ink to make the pollen readily visible. Flowers were emasculated only before selfing or crossing with self-compatible species in the study of activity of pollen in the style.

TABLE 1
PLANT COLLECTIONS ARRANGED ACCORDING TO SPECIES, REGION OF
COLLECTION, AND COLLECTOR'S DATA

Species	Region of collection			Collector	Collector's accession number	P. I. number	Date collected
	Locality	Province	Country				
<i>Lycopersicon</i>							
<i>peruvianum</i>	Pacasmayo	Libertad	Peru	B and T*	1009	126926	12/14/37
<i>L. peruvianum</i>	Pacasmayo	Libertad	Peru	B and T	1011†	126928	12/14/37
<i>L. peruvianum</i>	Chancay	Lima	Peru	B and T	1013	126930	12/14/37
<i>L. peruvianum</i>	Culebras	Ancash	Peru	B and T	1027‡	126944	12/15/37
<i>L. peruvianum</i>	Culebras	Ancash	Peru	B and T	1028	126945	12/15/37
<i>L. peruvianum</i>	Culebras	Ancash	Peru	B and T	1029	126946	12/15/37
<i>L. peruvianum</i>	San Jerónimo	Lima	Peru	Rick†	SAL 25		12/ 8/48
<i>L. peruvianum</i>	Cajamarquilla	Lima	Peru	Rick	SAL 28		12/12/48
<i>L. peruvianum</i>	Supé	Lima	Peru	Rick	SAL 41		1/29/49
<i>L. peruvianum</i>	Otusco	Libertad	Peru	Rick	SAL 55		2/18/49
<i>L. peruvianum</i> var.							
<i>dentatum</i>	Lluta Valley	Tarapaca	Chile	B and T	1231	128645	3/24/38
<i>L. p. dentatum</i>	Lluta Valley	Tarapaca	Chile	B and T	1232	128646	3/24/38
<i>L. p. dentatum</i>	Azapa Valley	Tarapaca	Chile	B and T	1233	128647	3/24/38
<i>L. p. dentatum</i>	Azapa Valley	Tarapaca	Chile	B and T	1234	128648	3/24/38
<i>L. p. dentatum</i>	Chacassilla	Tarapaca	Chile	B and T	1241	128655	3/25/38
<i>L. p. dentatum</i>	Tacna	Tacna	Peru	B and T	1243‡	128657	3/26/38
<i>L. p. dentatum</i>	Tacna	Tacna	Peru	B and T	1245	128659	3/26/38
<i>L. p. dentatum</i>	Tacna	Tacna	Peru	B and T	1247	128661	3/26/38
<i>L. p. dentatum</i>	Moquegua	Moquegua	Peru	B and T	1249	128663	3/27/38
<i>L. peruvianum</i>	Miraflores	Lima	Peru	B and T	1263‡	126431	11/23/37
<i>L. p. dentatum</i>	Hac.SanYsidro	Lima	Peru	Rick	SAL 33		12/29/48
<i>L. p. dentatum</i>	Cajacay	Ancash	Peru	Rick	SAL 39		1/29/49
<i>L. peruvianum</i> var.							
<i>humifusum</i>	San Juan	Cajamarca	Peru	B and T	1074	127829	12/23/37
<i>L. hirsutum</i>	Yaso	Lima	Peru	B and T	976-?-980	126444‡	11/24/37
<i>L. hirsutum</i> var.							
<i>glabratum</i>	Baños	Tunguragua	Ecuador	B and T	648	129157	4/21/38
<i>L. hirsutum</i>	Canta	Lima	Peru	Rick	SAL 11		10/25/48
<i>L. hirsutum</i>	Samne	Libertad	Peru	Rick	SAL 54		2/18/49
<i>L. hirsutum</i> var.							
<i>glabratum</i>	Baños	Tunguragua	Ecuador	Rick	SAL 60		3/ 8/49
<i>L. glandulosum</i>	Canta	Lima	Peru	Rick	SAL 12		10/23/48
<i>L. glandulosum</i>	Yura	Arequipa	Peru	Rick	SAL 30		12/23/48
<i>L. glandulosum</i>	Surco	Lima	Peru	Rick	SAL 49		2/13/49

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‡ The present study was concerned primarily with these collections.

CULTURAL METHODS

There is an advantage in using tomatoes in incompatibility research. Plants separated many generations can be crossed and tested against one another, because plants can be maintained indefinitely as clones.

Scoring fruit-set was at times a difficult matter. If growing conditions

were good, incompatibly pollinated flowers dropped as soon as unpollinated flowers, about six to nine days after anthesis. But if poor conditions prevailed, flower-drop was delayed. This necessitated waiting for appreciable enlargement of the compatibly pollinated ovaries (which was also delayed) before making the count.

Both cuttings and seedlings tended to cease flowering under the low light intensity of the dark months of November and December. It was discovered that by keeping the plants pot bound they could be forced into bloom earlier and their rate of vine growth reduced. Pot binding was found to be a more effective and less expensive method of guaranteeing flowering than artificial illumination. Accordingly, seedlings did best when they were started in three-inch pots then were transferred to four-inch pots when the first inflorescence had bloomed. If fertilized lightly at two-week intervals, they continued to flower in these pots for three or more months.

The plants whose growth was restricted in this fashion in general resembled *L. peruvianum* in its native habitat more than did plants given unlimited soil and water.

TESTS WITH *L. PERUVIANUM*

Preliminary investigations established that self-incompatibility is present in *L. peruvianum*. Several lines of this species were tested for self-incompatibility by caging and self- and cross-pollinating as previously described. The results are presented in table 2. Only one instance of cross-sterility was found (1231-1 \times 1231-2). Later, 1074-3 was found probably to be male sterile. Self-incompatibility and cross-fertility were demonstrated in all lines.

Subsequently, more extensive tests were made of self- and cross-compatibility in the following 12 collections of *L. peruvianum* and its varieties: 1009, 1027, 1074, 1243, 1263, SAL 25, 28, 33, 39, 41, and 55 (table 1). Self-pollinations were attempted in the greenhouse during the winter season on at least six flowers of each of ten or more plants of each collection. Additional flowers of each plant were pollinated with another plant of the same line. Less than five per cent of the selfed flowers developed fruits, of which the majority was parthenocarpic, the remainder containing one or two—rarely more—seeds apiece. In contrast, more than 95 per cent of the cross-pollinated flowers set fruits, nearly all of which had a full content of 40 to 50 seeds. On the basis of these tests, not one of the several hundred plants tested displayed even a moderate degree of self-compatibility.

SELF-INCOMPATIBILITY IN OTHER SPECIES OF *LYCOPERSICON*

Muller (1940) divided the genus *Lycopersicon* into two subgenera: The red-fruited species were classified under *Eulycopersicon*, and the green-fruited ones under *Eriopersicon*. The two species of *Eulycopersicon* are *L. esculentum* Mill., and *L. pimpinellifolium* (Jusl.) Mill.; these are self-fertile, as has been observed by many who have studied them. *Eriopersicon* contains *L. peruvianum* (L.) Mill., *L. cheesmanii* Riley, *L. hirsutum* Humb. and Bonpl., and *L. glandulosum* C. H. Mull. *L. cheesmanii*, a native of the Galapagos Islands, has evidently not yet been grown for experimental pur-

poses. The rest of the subgenus was tested during the course of this investigation.

Muller distinguished two varieties, *dentatum* and *humifusum*, as distinct from the typical form of the species *L. peruvianum*. *L. p. dentatum* was represented in nine different lines studied in the general survey of self-incompatibility in the species. The source of these plants and the results of

TABLE 2
SUMMARY OF COMPATIBILITY TESTS IN *L. PERUVIANUM**

<i>Lycopersicon peruvianum</i>				<i>L. peruvianum</i> var. <i>dentatum</i>			
Female parent	Male parent	Number of flowers	Number of fruit set	Female parent	Male parent	Number of flowers	Number of fruit set
†1009-2	Self	6	0	1231-1	Self	6	0
	1009-1	6	6		1231-2	4	0
1011-1	Self	8	0		1231-3	4	3
		1011-2	7	1232-1	Self	9	0
		1011-3	8		1233-1	7	7
1013-1	Self	7	0	1233-2	Self	14	0
	1013-2	3	3		1233-1	8	8
	1013-3	8	8		1233-3	7	7
1027-1	Self	9	0	1234-2	Self	11	0
	1027-2	5	5		1234-1	6	5
	1027-3	6	6		1234-3	6	6
1028-3	Self	8	0	1241-2	Self	8	0
	1028-1	13	12		1241-1	8	6
	1028-2	8	6		1241-3	8	6
	1028-4	6	6	1243-1	Self	10	0
	1028-5	5	5		1243-2	5	5
1029-2	Self	4	0		1243-3	5	4
	1029-1	7	6	1245-2	Self	11	0
<i>L. peruvianum</i> var. <i>humifusum</i>					1245-1	5	2
					1245-3	6	5
				1074-1	Self	6	0
1074-2	3	3	1247-2		8	3	
1074-3	3	0	1247-3		9	7	
				1249-1	Self	8	0
					1249-2	8	5
					1249-3	3	3

* Pollinations made on caged branches in the field.

† Blood's accession number (Table 1 gives the collector's data on these lines).

tests for incompatibility are given in tables 1 and 2. In addition, *L. p. dentatum* was one of the parents of the 410-family, and was one parent of the interspecific hybrid 4-1. There can be no doubt that *dentatum* is self-incompatible and that the same loci conditioning self-incompatibility in the species were involved in this variety. The other lines studied, and the *peruvianum* parent of the other interspecific hybrid, are typical forms of *L. peruvianum*.

L. peruvianum var. *humifusum* is self-incompatible, also, as the crossing

summary in table 3 shows. The test was not complete, but the *Nicotiana* scheme seems to apply here, for the presence of three or four groups was indicated. Plant No. 3 seemed to be male-sterile.

L. hirsutum grows very vigorously at Davis, but does not flower well in the summer. Luckwill (1943) reported behavior suggesting a day-length response, which may be the principal factor preventing flowering at Davis. When unpollinated in the field or greenhouse, *L. hirsutum* set no fruit at all. Several plants have been self- and cross-pollinated occasionally, over a period of years; all self-pollinations and certain cross-pollinations failed to set fruit. In other combinations, however, crosses have yielded fruits.

L. hirsutum f. *glabratum* consistently set fruit after both self- and cross-pollination in our tests, and untreated plants in the field were fairly fruitful

TABLE 3
INCOMPATIBILITY REACTIONS IN FAMILY 1074

<i>Lycopersicon peruvianum</i> var. <i>humifusum</i>								
Female parent— 1074	Male parent—1074							
	1	2	3	5	6	8	9	10
1.....	○*	×†	○	○	○?	×	×	..
2.....	..	○	○	×	×	×	×	..
3.....	×	×	○	○	×	×	×	..
5.....	×	..	○	○	..	×	×?	..
6.....	×	×	○	○	○	×	×	..
8.....	×	×	○	○	×	○	○	..
9.....	×	..	○	○	×	○	○	..
10.....	×	×	○	×	×	×	○	○

* ○ indicates sterility.

† × indicates fertile cross.

in contrast to those of the typical form of the species. Fruit was set with pollen from *L. hirsutum*, showing that pollen of the latter was functional. The use of *glabratum* pollen on *L. hirsutum*, however, failed to achieve fruit set.

Nine plants belonging to three different accessions of *L. glandulosum* (SAL 12, 30, and 49) were tested for self- and cross-compatibility. Selfs failed on all except one plant and nearly all of the crosses were fertile. The evidence for cross-sterility cannot be considered conclusive without further tests.

L. peruvianum has been grown experimentally at least since the time of Crane's (1915) report, and the green-fruited species in general have been subject to rather extensive investigation during the past fifteen years. It seems remarkable that, despite this long period and concentrated attention, self-incompatibility was not recognized until the work of Lamm (1950). Even more difficult to reconcile is the statement by MacArthur and Chiasson (1947) that "unfruitfulness of the more tropical green-fruited species does not appear to be caused by sterility factors, ploidy, major chromosomal aberrations, or oppositional factors." The apparent contradiction might be

explained by any one or several of the following reasons: 1) The plants do not grow well in some areas nor under certain conditions, and failure to produce fruit may have reflected merely a low level of vigor. 2) Even where adaptation was good, the delicate balance between the vegetative and flowering stages may have obscured the cause of unfruitfulness. 3) If the plants were fruitful, compatible plants were usually near and pollen vectors present. For experimental purposes several plants were usually grown; isolation of a single plant was rare. The responsibility of insect pollen vectors was likely to be overlooked; in the experience at Davis a surprisingly low level of vector activity accounted for a relatively high level of fruitfulness. 4) It is possible, though not likely, that in other investigations strains carrying self-fertility alleles were selected. 5) As discussed in the following section, certain conditions of growth favor pseudo-fertility or parthenocarpy.

Observations of the green-fruited species in their native habitat in Peru bear out these tests of the same species cultivated well outside their native range and in rather different environments. First, the amount of fruit-set varied according to the degree of activity of pollen vectors; the period of heavy set, which far exceeded anything we have observed in California, corresponded to the period of great vector activity, which again was at a very much higher level than that observed in California. The rates of vector visits to flowers of three green-fruited species and *L. esculentum* were observed to be many times higher at several sites in Peru than those in various localities in California; furthermore, many more species of bees belonging to six different orders were collected from tomato species in the former region (Rick, 1950).

Second, occasional plants that were well isolated by distance from others were less fruitful than those growing in colonies.

Third, limited tests of compatibilities of three green-fruited species in their native sites in the vicinity of Lima, Peru, indicated self-incompatibility and cross-compatibility. The rate of fruit-set of untreated exposed flowers was compared with that of flowers that were untreated, others selfed and others cross-pollinated within small isolation cages constructed of plastic screening. According to the results presented in table 4, no untreated flowers opening in cages set fruit, while a very high proportion of exposed flowers opening during the same period set fruit, despite the fact that for *L. glandulosum* and *L. hirsutum* it was not the period of greatest vector activity. That conditions within cages were not physiologically unfavorable for fruit-set is demonstrated by the high level of set by flowers that were artificially cross-pollinated within cages. The number of flowers selfed was too small to be very meaningful, yet all except two failed to set fruit, the two occurring on the same plant. At the time of observation these two fruits appeared subnormal in size, but it was not possible to examine them later to count the number of seeds that might have developed. Thus, although the possibility must be granted that one plant might have been genetically self-fertile, all other evidence points toward a condition of self-incompatibility in these species in their native habitat.

Self-fertility, well known in *Eulycopersicon*, has here been demonstrated in *L. hirsutum* f. *glabratum*. *L. peruvianum* and its varieties *dentatum* and

TABLE 4
TESTS IN PERU OF THE COMPATIBILITIES OF THREE
GREEN-FRUITED SPECIES

Species	Location	Exposed flowers untreated				Flowers in cages									
		Untreated				Self-pollinated				Cross-pollinated					
		Number of plants tested	Number of flowers	Number of fruits set	Per cent of fruits set	Number of flowers	Number of fruits set	Per cent of fruits set	Number of flowers	Number of fruits set	Per cent of fruits set	Number of flowers	Number of fruits set	Per cent of fruits set	
<i>L. peruvianum</i> ...	San Jerónimo...	5	345	299	87	185	0	0	17	15	88	
<i>L. glandulosum</i> ...	Surco.....	5	60	43	72	66	0	0	24	2	8	14	12	86	
<i>L. hirsutum</i>	Surco.....	4	76	49	65	55	0	0	5	0	0	5	2	40	

humifusum, *L. glandulosum* and *L. hirsutum* of the *Eriopersicons* have been shown to be self-incompatible.

INHERITANCE OF SELF-INCOMPATIBILITY

In order to study the character as it appears in nature, studies of inheritance of self-incompatibility in *L. peruvianum* were limited to a single geographic collection. Plants of Blood's accession No. 1011, P.I. No. 126928, were used. Two plants were crossed, and a progeny of 56 plants was grown and tested. This progeny was given the family number 101.

TABLE 5

RESULTS OF POLLINATIONS IN ALL POSSIBLE COMBINATIONS OF
12 PLANTS OF FAMILY 101 (1011-1 × 1011-2)*

Female parent	Male parent											
	1	3	5	4	8	10	9	28	34	7	14	40
1	0/8	0/7	0/8	4/6	2/2	3/3	2/2	2/3	4/4	4/4	4/4	4/4
3	0/11	0/9	0/8	4/4	3/3	2/3	3/3	3/3	3/3	4/4	4/4	4/4
5	0/7	0/12	4/22	6/9	6/7	2/6	2/7	4/4	3/3	4/4	4/4	4/4
4	3/3	3/3	9/9	0/10	0/3	0/2	2/2	3/3	2/2	1/4	1/4	1/4
8	4/4	3/4	4/6	0/3	0/23	0/2	2/2	3/3	3/3	4/4	4/4	4/4
10	4/6	6/7	5/11	0/4	0/3	3/14	2/2	2/3	2/2	4/4	4/4	4/4
9	4/8	3/3	5/9	3/3	2/2	4/5	0/19	0/4	0/4	4/4	4/4	4/4
28	2/3	3/3	2/2	3/3	2/2	2/2	0/2	0/32	0/3	4/4	4/4	4/4
34	3/3	3/3	3/3	3/3	3/3	2/2	0/2	0/4	0/30	4/4	4/4	4/4
7	3/3	3/3	3/3	3/3	3/3	2/2	2/2	3/4	3/3	0/10	0/3	0/4
13	6/9	8/9	6/6	6/9	10/11	6/6	6/6	3/3	3/3	0/4	0/11	0/4
40	3/3	3/3	3/3	3/3	6/6	4/4	5/5	2/3	3/4	0/5	0/6	0/10

* Numerator = number of fruits set.

Denominator = number of flowers pollinated.

In testing the plants, crossing in all possible combinations would have been a formidable task ($56^2 = 3136$). Consequently, all 56 plants were crossed with eight selected plants as test pollen parents. Four groups were found: one group was sterile with plant Nos. 1, 3, and 5; another was sterile with plant Nos. 2, 4, 8, and 10; a third was sterile with plant No. 9 and a fourth fertile with all pollen parents. Using two plants of the third group and three of the fourth for another round of pollinations, it was found that plants of either group would not cross within the group, but would cross freely with other groups. Therefore each pollen parent was sterile in pollinations on plants of its own group, but fertile with three other groups. Results of pollinations in all possible combinations of twelve test pollen parents are presented in table 5, which shows the typical number of flowers pollinated and fruit set during the various phases of the study.

All 56 plants were crossed reciprocally with both 1011 parents. All set fruit freely.

The 56 plants of the 101 family were grown under the best conditions possible, as described in the section on cultural methods. With only a few

exceptions, the plants flowered well, set fruit well, and seemed disease-free. The very high degree of isolation is indicated by the facts that no fruits set on unpollinated flowers, and that no insects were seen in the greenhouse during the course of the pollinations. Reactions were precise. It was possible to score results reliably within a week.

These pollinations are summarized in table 6. Since the four groups vary in size from 11 to 17 plants, a Chi-square test was applied to determine if the segregation could safely be assumed to be 1:1:1:1. The deviation from expected values is not significant ($X^2 = 1.858$, 3df, $p = .5 - .7$).

These tests showed that every plant in the 101 family of 56 could be classified into one of four groups of equal size. Crosses within groups were

TABLE 6
FAMILY 101 CLASSIFIED ON THE BASIS OF TEST POLLINATIONS

Female parents*	Male parents: Siblings used as test parents				Parents	
	1, 3, 5: A	2, 4, 8, 10: B	9, 28, 34: C	7, 13, 40: D	1011-1	1011-2
A.....	○	×	×	×	×	×
B.....	×	○	×	×	×	×
C.....	×	×	○	×	×	×
D.....	×	×	×	○	×	×

* Group	Plant numbers of female parents	Total
A:	1, 3, 5, 14, 15, 19, 21, 23, 25, 26, 33, 35, 39, 47, 53, 55.....	16
B:	2, 4, 8, 10, 16, 17, 18, 24, 27, 30, 32, 36, 37, 42, 44, 45, 51.....	17
C:	9, 11, 12, 20, 22, 28, 29, 34, 43, 46, 48, 52.....	12
D:	6, 7, 13, 31, 38, 40, 41, 49, 50, 54, 56.....	11
		56

not successful, but plants of different groups could be crossed freely. All plants were compatible with each parent.

Inheritance of this type is expected under the *Nicotiana* scheme (East and Mangelsdorf, 1925) if each of the parents brought two different alleles into the cross. Since it might also be expected under other schemes, other tests were conducted to verify the pattern of inheritance. Advantage was taken of the ease of clonal propagation of tomatoes in order to mate plants in segregating generations with F_1 's and parents.

The following progenies were grown: (1) 101-5 \times 101-6, (2) 101-6 \times 101-5, (3) 101-5 \times 101-8, (4) 101-8 \times 101-5, (5) 101-6 \times 1011-2, and (6) 101-6 \times 1011-1. These crosses and their progenies are presented in table 7.

Both progenies (1) and (2) of plants 5 and 6 segregated into four inter-fertile, intrasterile groups, of which one was sterile with one parent and another sterile with the other parent. The other two classes were fertile with both parents; all were fertile with both F_1 's.

Both progenies (3) and (4) of plants 5 and 8 segregated into two inter-fertile, intrasterile groups, one of which was sterile with the staminate F_1 , while the other was sterile with the staminate parent.

The backcross progenies of plant 6 (5) and (6) segregated into two classes, one of which was sterile with the staminate F_1 parent, the other fertile with both F_1 's.

The cultural conditions under which the plants were grown at the time of the testing of these progenies were good; the results should be as valid as those of the tests of the parent 101 plants.

According to the capsella type of incompatibility as reported by Riley (1936) a population could be composed at the most of only three intrasterile, interfertile groups, and crosses between different plants of any two groups

TABLE 7
INHERITANCE OF STERILITY RELATIONSHIPS IN *L. PERUVIANUM*

Cross	Parent	Progeny	Number of plants	Genotypes	Tested sterility relationships
(original)	1011-1 \times 1011-2	101-A	16	S_1S_3	No intergroup sterility
		B	17	S_1S_4	
	$S_1S_2 \times S_2S_4$	C	12	S_2S_3	
		D	11	S_2S_4	
(1)	101-5(A) \times 101-6(B)	50-A	6	S_1S_2	50-A is sterile with 1011-1, D with 1011-2
		B	2	S_1S_4	
	$S_1S_3 \times S_2S_4$	C	4	S_2S_3	
		D	8	S_2S_4	
(2)	101-6(B) \times 101-5(A)	52-A	4	S_1S_2	52-A is sterile with 1011-1, D with 1011-2
		B	5	S_1S_4	
	$S_2S_4 \times S_1S_3$	C	3	S_2S_3	
		D	5	S_2S_4	
(3)	101-5(A) \times 101-8(B)	53-A	5	S_1S_4	53-A is sterile with 101-8 and 50-B; B with 1011-2 and 50-D and 51-B
	$S_1S_3 \times S_1S_4$	B	3	S_2S_4	
(4)	101-8(B) \times 101-5(A)	51-A	8	S_1S_3	51-A is sterile with 101-5, B with 1011-2, 50-D and 53-B
	$S_1S_4 \times S_1S_3$	B	12	S_2S_4	
(5)	101-6 \times 1011-2	55-A	4	S_2S_3	55-B is sterile with 1011-2
	$S_2S_4 \times S_2S_4$	B	6	S_2S_4	
(6)	101-6 \times 1011-1	56-A	4	S_1S_2	56-A is sterile with 1011-1
	$S_2S_4 \times S_1S_2$	B	6	S_1S_4	

should yield different results. One group was composed of more than one genotype. Although all reciprocal crosses yield the same number and type of groups, as found here, the capsella scheme obviously does not apply to these data.

Kakizaki, in 1930, reported an exhaustive search for the basis of the incompatibility reactions found in cabbage. He found that: 1) the progeny of two plants might contain more than four genotypes; 2) when a progeny segregates into two classes both may be fertile with both parents; and 3) two sister plants which are cross-sterile may be of different genotypes. To explain these and other facts, he proposed a series of *S* factors which act in

the *Nicotiana* manner, but which are modified by a series of *T* factors which favor fertilization, yet are not alleles of the *S* factors. He called this the associate type of incompatibility. The present data cannot fit this scheme, because no more than four groups were found in the progeny of any two plants. No progenies composed of two groups were found where both groups were fertile with both parents.

Nor does the behavior of *L. peruvianum* resemble that of *Crepis* or *Parthenium* as reported recently (Hughes and Babcock, 1950; Gerstel and Riner, 1950): crosses between compatible plants which may produce four types of offspring do not always produce the same four types; and incompatibility may be found between groups which are not genotypically identical. Also striking is the difference which often exists if the cross is made reciprocally. This occurs in the *Nicotiana* scheme only when homozygotes are produced by bud pollination or other methods of altering normal behavior. The *Nicotiana* scheme alone fits the *L. peruvianum* data.

PSEUDO-FERTILITY

This term, first used by East and Park (1917), has provoked dismay and discussion in nearly every paper written on the subject of self-incompatibility. It is so commonly found that for a long time Stout (1916, 1923, and later) felt that the incompatibility gene was extremely mutable. This trouble interfered with the early work of East and Park, Stout, Riley, and many other workers, including the writers (cf. 101-5 selfed and 101-10 selfed in table 5).

In the present experiment it was observed that in one instance selfed fruit had set just after pruning the plant. Accordingly, two comparable plants were chosen; four branches on each were caged in the manner described on page 102, "Materials and Methods." All fruits were removed from one plant: 10 main branches, bearing 128 fruitful inflorescences with about 10 fruits each. From the four branches below the cage 21 fruitful inflorescences were removed. After a time, 25 flowers had opened inside the cage. These were self-pollinated, but none set fruit. The plant soon stopped flowering, but flowers again appeared later in the season. The other plant was left intact, and 22 flowers which opened inside the cage were self-pollinated. None of these set any fruit; this plant did not cease flowering.

In another case, it was noted that a few fruits were set without pollination following a compatible pollination of a number of other flowers on the same inflorescences. A parallel experiment was set up, using two plants as females and one as male. The cross on one female was compatible, on the other, incompatible. On both plants all the flowers on one branch of each inflorescence were pollinated. The results again were negative. Only one of 96 unpollinated flowers set a fruit, which appeared on a plant that had been compatibly pollinated. It therefore appears that pollination of a flower on one branch of an inflorescence does not stimulate pseudo-fertile fruit-set on the other branch. This experiment was carried out in the screenhouse, and serves to indicate the efficiency of isolation obtained there, as well as the dependability of the technique employed.

A plant growth substance was utilized in another effort to set fruit by

self-pollination. Eyster (1941) reported very successful use of 10 ppm of alpha naphthalene acetamide to overcome self-incompatibility in *Petunia*. Mann and Minges (1949) have recommended 25–100 ppm of the sodium salt of para-chloro-phenoxy-acetic acid in water for producing fruit on unpollinated *L. esculentum*. A number of incompatible crosses were made in *L. peruvianum* and inflorescences were sprayed in various ways with a 100 ppm solution. The only effect of the treatment was to prevent the abscission from the inflorescence of flowers which would normally have dropped. These flowers shriveled and became yellow; though dead, some remained on their pedicels for several weeks.

Although compatibly pollinated fruit set normally and were not injured by the spray, it was felt that a stronger solution would cause injury effects so great as to obscure any beneficial results obtained; Mann and Minges (1949) reported serious injury at a concentration of 250 ppm.

The fact that there was no injury to the flowers and that the flowers remained on the pedicels long after normal abscission suggests that in this species the incompatibility reaction is not merely the result of a reduction of the rate of growth of the pollen tubes, but more likely a complete cessation of this growth.

Contrary to the results of the preceding experiments, pseudo-fertility was observed under conditions which, though not planned, were nonetheless rather clearly defined. In the late greenhouse season at Davis, particularly in May, open-pollinated (presumably selfed) flowers tend to set small fruits with a few seeds each. Likewise, Bohn (1948) obtained open-pollinated fruit in the greenhouse late in the spring after a winter of unfruitfulness. According to our observations, the setting of fruit after incompatible pollinations seems to require a level of vigor slightly below that at which the incompatibility reactions are effective and does not seem to be affected by length of day or light intensity. In this connection it should be noted that the balance of physiological conditions within the *peruvianum* plant seems extremely precise. The removal of a few roots growing through the pot is often sufficient to shift the plant quickly from a vegetative phase in which all inflorescences are aborted at an early stage to an abundant flowering phase.

In their native habitat, plants of *L. peruvianum* grow in very arid areas, in some places receiving moisture only from the heavy winter fogs in the "loma" belt. They grow as long-lived perennials, sending up new vigorous shoots during the moist period and dying back to the crown shortly thereafter. At some stage during the gradual drying of the soil, the plants might reach a condition comparable with that of the pot-bound plants that gave the pseudo-fertile reaction in our tests; in fact, the latter show much greater morphological resemblance to plants in their native habitat than plants in lush growth in the field in California. It seems possible that, as the soil dries out after the short wet season, the plants might pass through successive stages of normal incompatibility, pseudo-fertile fruit-set (which might serve as a device to assure seed setting in the absence of vectors or of nearby compatible plants), and then complete unfruitfulness as the previously set fruits are matured.

BUD POLLINATION

In *Nicotiana* (East, 1929), cabbage (Pearson, 1932), and many other plants, incompatible self- and cross-pollinations have been induced to set fruit by pollinating a stigma one to several days prior to anthesis. East states, after describing the process, "it further follows, therefore, that the inhibiting substance or reaction does not occur, or is not so effective, while the flower is in the bud stage."

In the present experiment, frequently buds show a stigma considerably exerted beyond the tip of the flower. In such cases bud pollination can be performed readily and without any damage to the flower. Hundreds of pollinations of buds were made in connection with cross- and self-pollinations of open flowers. Only two fruits were produced by buds where incompatible pollinations of flowers produced none. Both fruits were parthenocarpic, containing small aborted ovules.

Even if the immature flower is forcibly opened and a portion of the anther column split, no reaction occurs in the bud different from that occurring in the flower.

Buds of *L. peruvianum* open about one per day; tomorrow's bud is turning yellow today, the bud to open the next day is still greenish. This second bud is receptive in about half the cases. Very rarely is any fruit obtained from compatible pollinations on any buds younger than this.

It was noted that styles tend to be exerted as much in Peru as they are here. Therefore, if bud pollination were successful the value of incompatibility as a device to guarantee complete cross-pollination might be lost, for insect vector activity is so great that few buds would escape pollination before the flower opened.

Bud fertility has not been found in *Oenothera organensis* (Emerson, 1940) Guayule (Gerstel and Riner, 1950), nor in any of the species investigated in the present experiment. Not enough evidence has been presented to justify a formal statement, but there is a possibility that bud fertility might distinguish certain species in which the relative rate of growth of pollen tubes determines whether fertilization shall succeed from those species in which there is a complete inhibition of incompatible tubes.

FERTILITY RELATIONS OF SPECIES HYBRIDS

After it became certain that *Lycopersicon peruvianum* is self-incompatible and that the inheritance of this condition follows the *Nicotiana* scheme, it was logical to test the inheritance in species hybrids, which were available for this work.

Hybrids between *L. esculentum* and *L. peruvianum* have been very difficult to produce. With certain strains of *L. peruvianum* it has been necessary to resort to embryo culture techniques (Smith, 1944). The hybrid produced by Smith has been maintained by cuttings and in this experiment is numbered 4-1. Its parents were the *L. esculentum* variety Michigan State Forcing and the *L. peruvianum* line 1243 of Blood's collection. All interspecific crosses are made with *L. esculentum* as pistillate parent, for *L. esculentum* pollen fails to set fruit on *L. peruvianum*.

In 1945, 15 clonal plants from Smith's hybrid (4-1) were set out in isolation to obtain a measure of its fruitfulness. During the season an estimated 100,000 flowers were produced, from which 350 fruits were obtained. The total seed yield was about 150, of which 70 germinated. This reproductive rate is 0.0007 offspring per flower, as compared with 50 to 100 offspring per flower normal in either species parent.

Although chromosome behavior at meiosis in inter-sub-generic hybrids is usually normal (MacArthur and Chiasson, 1947) all tend to be sterile. MacArthur and Chiasson suggested that hybrid sterility has its basis in cryptic structural hybridity, or in genic influences. J. W. Lesley (1948) confirmed these opinions, but pointed out that the possible genic influences have not yet been investigated. M. M. Lesley (1950) reported the first case of a chromosomal inversion—the only aberration found—in a hybrid of *L. esculentum* and *L. peruvianum* and discussed the role of this aberration in reducing fruitfulness. This hybrid was produced by J. W. Lesley, using the method of Smith (1944). Its parents were the *L. esculentum* variety Pearson and an *L. peruvianum* plant of Blood's collection, Acc. No. 1027. The *L. peruvianum* parent is typical of the species, being low growing, fruitful, and vigorous at Davis. This parent was numbered 4-37, and its hybrid offspring was numbered 4-36 upon receipt at Davis. Field tests of the fertility of 4-36 have been made; these and hundreds of controlled self-pollinations have failed to set any seedy fruit.

As soon as 4-36 came into bloom, it was crossed with 4-1. It was found that either hybrid set fruit freely with pollen of the other; nearly every cross-pollinated flower set fruit. The seed set was only about 10 per cent of that of either parent species (5 to 15 seeds against 50 to 250) but it was at least 10,000 times as fertile as 4-1 self-pollinated. Fruit-set was very good; if the pistillate parent was vigorous, every pollinated flower usually produced a fruit.

We are aware of reference in the literature to only one other case of a self-incompatible hybrid between a self-compatible species and a self-incompatible species. Anderson and De Winton (1931) reported that the F_1 between *Nicotiana langsdorfii* and a certain plant of *N. alata* contained half self-fertile and half self-sterile plants, where normally all such plants would be self-fertile. The *N. alata* plant itself had been exceptional, for it was compatible as a male with *N. langsdorfii*, but incompatible as a female. The self-sterile F_1 plants performed similarly with *N. langsdorfii*, but were fully fertile with *N. alata*. They were cross-sterile with their self-fertile parent but cross-fertile with their self-sterile parent. This behavior was explained as the result of the presence in the exceptional *N. alata* parent, of the gene S_F , which was allelomorphic with S_1, S_2, S_3, S_4 , et cetera. Gene S_F showed the same behavior as other S alleles, that is, its presence in the style inhibits the growth of pollen carrying the same factor, but S_F in addition, inhibits pollen carrying the self-fertility allelomorph, S_1 .

L. esculentum \times *L. peruvianum*, then, is the second case of this sort, but appears not to result from a single mutant gene in one species.

In degree, the *esculentum* \times *peruvianum* hybrids are as self-incompatible as their incompatible parents. It seems that incompatibility, not hybrid

sterility due to chromosomal or genic unbalance explains most of the unfruitfulness of the selfed F_1 . Having demonstrated this, use of these hybrids in further testing of incompatibilities is legitimate because viability of pollen and ovules is shown not to be a limiting factor in fruit-set. Hybrid sterility appears to cause the reduced seed set noted above, however.

The cross-pollination of many flowers attempted on many occasions reveals the following relations between parents and F_1 hybrids. *L. esculentum* sets fruit freely after being pollinated by *L. peruvianum*, but ovules are usually aborted at an early stage. *L. peruvianum*, on the other hand, does not set fruit with pollen of *L. esculentum*, although this cross has been at-

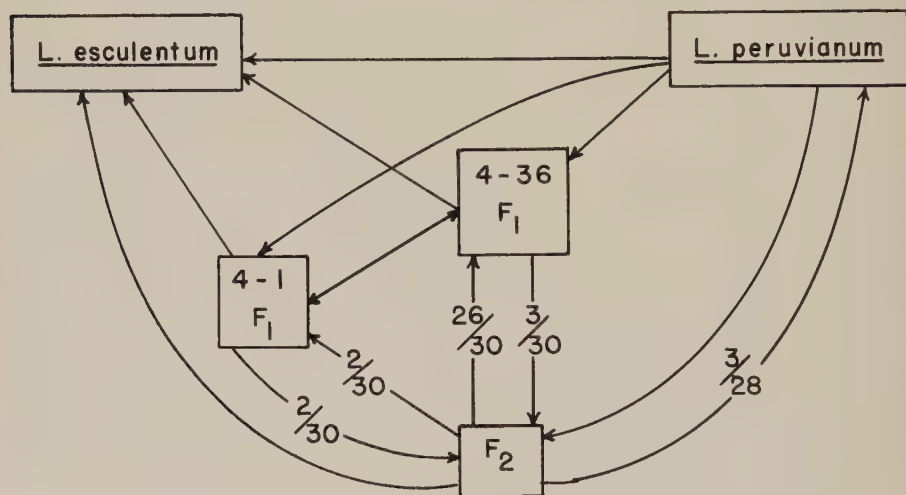


Figure 1. Pollination relations of species and their hybrids. Direction of the arrow indicates the only direction in which the cross can be made; the arrow leads from the pollen to the seed parent. Fractions indicate the proportion of plants in the F_2 that yielded fruit. Arrows without fractions indicate successful pollinations in all combinations.

tempted many times. Pollen of the F_1 hybrids sets fruit readily on the *esculentum* parent, but pollen of the latter does not set fruit on the F_1 . Also, pollen of the *peruvianum* parent sets fruit on the hybrids, but the reciprocal cross fails.

Thirty plants of the F_2 population growing in the field were used for tests of compatibility. Crosses in all possible combinations were made between these and the following lines: *L. esculentum* var. Pearson as a staminate parent and *ms*₁₆ var. Pritchard as pistillate parent, a plant (454-1) of an unrelated line (collection 1009) of *L. peruvianum*, and the two F_1 hybrids, 4-1 and 4-36. Flowering branches of the F_2 segregates were isolated in small cheesecloth cages, while the other parents were isolated by distance from other tomato plantings. The failure of untreated flowers to set in all test plants testifies to the effectiveness of the isolation measures. The results of these test crosses are presented in table 8.

The following salient facts were revealed by these test crosses. All 30 tested plants of the F_2 were self-incompatible. All set fruit when used as

pistillate parents in crosses with *L. peruvianum* while only three were successful staminate parents in the reciprocal crosses. Matings with *L. esculentum* were even more decisive, for all crosses between this species as the pistillate parent and the F_2 segregates succeeded, while all of the reciprocals failed. Since all of the fruits set in these tests contained seeds, neither pollen nor ovule sterility could possibly have accounted for any of the cross failures with the F_2 plants.

Neither of the F_1 parents was very potent as staminate parent in these crosses, for only two of the 30 F_2 plants set fruit after pollination with 4-1, and only three of 30 with 4-36. The reciprocal crosses differed greatly, for 26 of the 30 F_2 plants were effective as pollen parents on 4-36, while only two succeeded on 4-1.

All of the pollination relations between the two species and their F_1 and F_2 hybrids are summarized in figure 1.

INHERITANCE OF SELF-INCOMPATIBILITY IN THE SPECIES HYBRIDS

Although the inheritance pattern might be explained in several ways, the simplest hypothesis is based on action of the self-incompatibility alleles, acting according to the following assumptions. *L. esculentum* possesses a recessive fertility allele, s_e , at the s locus. In the combination $S_x s_e$, S_x is dominant in the sense that the reaction of the style is governed by S_x and not s_e . All tested S alleles from *L. peruvianum* oppose the allele from *L. esculentum* in addition to the same S genes from *L. peruvianum*. It follows then that two hybrids with *peruvianum* lines that carry different S alleles would each be self-incompatible but could cross with each other. All of the compatibility relations between the parental species and the two F_1 hybrids as presented in figure 1 can thereby be explained.

According to our hypothesis, all of the F_1 hybrids that could be obtained from a single plant of *L. peruvianum* and any plants of *L. esculentum* should belong to only two incompatibility groups. Additional data that have been obtained with different material bear out this expectation. Six hybrids have been obtained between *L. esculentum* and accession LA143, a green-fruited tomato species collected as No. 30349 by the Third Expedition to the Andes by the University of California Botanical Garden. This line, although it fits botanical descriptions of *L. peruvianum* var. *dentatum*, will not hybridize with other collections of that species and is therefore provisionally labelled *L. chilense* (Rick and Lamm, unpub.). This species is also self-incompatible according to the *Nicotiana* scheme (Lamm unpub.) and likely carries S alleles at the same locus. Compatibility tests in all combinations of the six hybrids classified them precisely into two intraincompatible and intercompatible groups of three hybrids each. While this sample is too small to rule out statistically the possibility that other compatibility groups might exist, it is at least in complete agreement with the prediction of our hypothesis.

This scheme demands that the F_2 be composed of only two genotypes: $S_1 S_3$ and $S_3 s_e$ (assuming that the *peruvianum* parents were $S_1 S_2$ and $S_2 S_4$, the pistillate F_1 plant $S_1 s_e$ and the staminate F_1 plant $S_3 s_e$). The presence

of an *S* allele in both genotypes requires that all of the F_2 be self-incompatible; this expectation is realized, for each of the 30 tested F_2 segregates failed to set fruit after selfing.

If all F_2 plants bear dominant *S* factors, none would be expected to set fruit when pollinated by *L. esculentum*, whereas all reciprocals should suc-

TABLE 8
RESULTS OF DIRECT AND RECIPROCAL CROSSES BETWEEN THE
 F_2 POPULATION AND VARIOUS TESTERS*

F_2 plant no.	Testers used as pistillate parents				Testers used as staminate parents					
	<i>L. peru- vianum</i>	<i>L. escu- lentum</i>	4-1 F ₁	4-36 F ₁	<i>L. peru- vianum</i>	<i>L. escu- lentum</i>	4-1 F ₁	4-36 F ₁	Self	Unpol- linated control
1	5N	3F, 2N	6N	1F, 5N	4F, 2N	6N	6N	6N	6N	6N
4	1S, 4N	5F, 1N	6N	2F, 4N	6F	2P, 4N	3P, 3N	2P, 4N	6N	3P, 3N
7	5N	5F, 1N	4N	3F, 3N	4F, 2N	6N	6N	6N	6N	6N
10	5N	5F	6N	3F, 3N	5F, 1N	6N	6N	6N	6N	6N
12	2N	5F	6N	2F, 1S, 3N	6F	6N	6N	6N	6N	6N
15	5N	5F	6N	1F, 3N	6F	6N	1F, 1S, 4N	6N	6N	6N
18	5N	5F	6N	3F, 3N	5F, 1S	6N	6N	5F, 1N	6N	6N
19	..	5F	6N	2F, 4N	6F	6N	6N	6N	6N	6N
22	5N	5F	6N	6N	5F, 1N	1P, 5N	1F, 1S, 4N	6N	6N	6N
25	2S, 3N	6F	1F, 5N	6N	6F	6N	6N	6N	6N	6N
28	5N	5F, 1N	6N	6N	6F	6N	6N	6N	6N	6N
30	..	5F	6N	1F, 1P, 4N	2F	4N	4N	3F, 1N	4N	6N
33	1F, 4N	6F	4N	5F, 1S	4F	6N	3S, 3N	6N	4N	6N
36	5N	5F	6N	2F, 4N	5F, 1N	6N	2F, 4N	6N	6N	6N
39	5N	6F	6N	5F, 1N	6F	2P, 4N	3P, 3N	3P, 3N	4P, 2N	3P, 3N
41	5N	3F, 1N	6N	1F, 5N	5F, 1N	6N	6N	6N	6N	6N
44	1P, 2S, 2N	6F, 1N	6N	1F, 5N	3F, 1N	4N	4N	4N	4N	4N
47	5N	4F, 1N	6N	5F, 1N	6F	1P, 5N	6N	3P, 3N	1P, 5N	4S, 2N
50	5N	5F, 1N	6N	2F, 4N	3F, 3N	6N	6N	6N	6N	6N
52	5N	5F, 1N	6N	1F, 5N	6F	3P, 3N	6N	1P, 5N	3P, 3N	2P, 4N
55	1P, 1S, 3N	5F	6N	2F, 4N	6F	1P, 5N	6N	6N	6N	6N
59	2S, 3N	1F, 2N	6N	5F, 1N	5F, 1N	5P, 1N	2P, 4N	1P, 5N	3P, 3N	3P, 3N
63	1S, 4N	3F, 1N	6N	6N	6F	6N	6N	6N	6N	6N
67	4F, 1N	5F, 1N	4F, 2N	3F, 3N	6F	6N	6N	6N	6N	6N
70	5N	4F	6N	3F, 3N	6F	6N	6N	6N	6N	6N
72	5N	5F	6N	1F, 5N	3F, 1N	6N	6N	6N	6N	6N
75	5N	5F, 1N	6N	4F, 2N	4N	4N	4N	4N	4N	4N
78	5N	4F	6N	1F, 5N	5F, 1N	6N	6N	4F, 2N	6N	6N
81	2F, 3N	4F, 1N	6N	1F, 5N	5F, 1N	6N	6N	6N	6N	6N
85	3S, 2N	6F	4N	1F, 5N	4F, 2N	6N	6N	6N	6N	6N

* F = Fruit with seeds
P = Parthenocarpic fruit
S = Calyx stimulated to remain, no fruit set
N = No fruit set, calyx shed

ceed. Here again, observations agree (table 8). The hypothesis further implies that all F_2 individuals should be fertile to pollination by the *peruvianum* parent since the latter likely bears a different *S* allele from those present in the F_2 . The results (table 8) again fit this expectation.

In respect to the crossing relationships between the F_2 and the two F_1 hybrids, results tend to deviate from expectations. It should be noted here that reactions are less clearly defined in these matings and are accordingly more difficult to interpret. The proportion of flowers setting fruit in compatible matings, for instance, instead of being uniformly high as in the previous

data, varies from low to high levels. The success of nearly all crosses (26 of 30) of $4-36 \text{ } \text{F}_2 \text{ } \text{S}_1\text{S}_e \times \text{F}_2 \text{ } \text{S}_3\text{S}_e$ agrees relatively well with the expectation of complete compatibility ($\text{S}_1\text{S}_e \times \text{S}_1\text{S}_3$ and $\text{S}_1\text{S}_e \times \text{S}_3\text{S}_e$), but in the reciprocal only two crosses of the 30 succeeded, whereas approximately half would be expected to succeed ($\text{S}_3\text{S}_e \times \text{S}_1\text{S}_e$). Approximately half of the crosses of $4-1 \text{ F}_1 \text{ (S}_3\text{S}_e) \text{ } \text{F}_2 \text{ (S}_1\text{S}_3) \text{ } \text{S}_1\text{S}_3$ should succeed and all of the reciprocal should fail. Actually, in both direct and reciprocal crosses two of 30 crosses set fruit. These tests with $4-1$ as a pistillate parent may not be very reliable, however, since by the time these crosses were made the clone had become so weakened by accumulated virus diseases that it set fruit poorly in crosses even with $4-36$ and plants of *L. peruvianum*.

In addition to the discrepancies noted above, it must be pointed out that behavior in these and other tests requires additional modifications of the hypothesis. In the first place, plants of five unrelated collections of *L. peruvianum* failed to set fruit as pistillate parents in crosses with either F_1 hybrid, yet crosses of this type ($\text{S}_x\text{S}_y \times \text{S}_1\text{S}_e$ and $\text{S}_x\text{S}_y \times \text{S}_3\text{S}_e$) should succeed, unless the extremely unlikely situation obtained that each of the collections possessed *S* alleles in common with both F_1 hybrids. An additional contradiction is provided by the cross of unrelated *L. peruvianum* $\times \text{F}_2$, for, according to the prediction ($\text{S}_x\text{S}_y \times \text{S}_1\text{S}_3$ and $\text{S}_x\text{S}_y \times \text{S}_3\text{S}_e$) all crosses should succeed, while actually only three of 28 were successful.

It appears from these observations that, in addition to the action of the *S* alleles, the hybrids inherit something from *L. esculentum* that renders their pollen impotent on the styles of *L. peruvianum*. If a single *esculentum* gene conditioned this inhibition and acted sporophytically in the respect that it affected all pollen whether it be homozygous or heterozygous, 25 per cent of the F_2 should be successful in pollinations on an unrelated plant of *L. peruvianum*. Although the observed proportion (3/28) does not deviate significantly from this expectation ($\chi^2_{25:3} @ 3:1, 1 \text{ df}, p = .10-.05$), two independent genes having similar effects provide a much better fit ($\chi^2_{25:3} @ 15:1, 1 \text{ df}, p = .50-.30$).

This additional assumption still does not satisfactorily explain the crossing relations observed between the F_2 and the two F_1 's, although, as already pointed out, the weakness of the hybrid $4-1$ might render the data untrustworthy. In view of the hybrid sterility of some of the segregates and the tremendous possibilities for modification of incompatibility reactions in hybrids of species that differ so greatly in genotype, it might be unreasonable *a priori* to expect to find complete agreement with hypotheses of such a simple nature. It is obvious that larger F_2 samples need to be tested not only with the lines used in these experiments but also in sib matings and that it might be necessary to reckon with different grades of compatibility.

BEHAVIOR OF POLLEN TUBES IN THE STYLE

In order to determine the approximate rate of growth of pollen tubes in the style (*L. peruvianum* compatibly pollinated), a number of styles were pollinated. At intervals of four hours, styles were removed from three flowers, each from a different inflorescence. Up to the 28-hour period, all flowers so treated later dropped without setting fruit. One of the flowers

whose style was removed at 28 hours produced a fruit. In the 32-hour group, two flowers set fruit, and all subsequent removal of styles failed to prevent fruit-set. In a second hourly experiment, the time was narrowed to 30 to 31 hours, after which style removal did not affect fruit-set.

These findings facilitated further study of pollen tube behavior in the style, for styles for cytological examination were picked 18 to 24 hours after pollination.

In studying the behavior of pollen tubes in the style, the methods of the following workers were tried: Chandler (1931), Nebel (1931), the triple stain of prepared sections as used by East and Park (1917) and Sears (1937). Sears' technique gave results which were comparable with those finally achieved, but the method is rather more time consuming than that eventually adopted. His methods as described for both *Geranium* and *Petunia* were tried; it was the latter which gave good results.

The method finally adopted was as follows: stigmas were pollinated in the customary manner, and after 18 to 24 hours the whole flower was picked, put into a small paper sack, and placed in a refrigerator. After a few hours or overnight the flowers had wilted. Occasionally, freshly picked styles were used, but slight wilting permitted easier handling. Styles were removed from the flowers and placed dry on a slide. It was found that by clamping the style lightly between two slides it could be held straight while it was split lengthwise with a hollow-ground microtome knife. A drop of lactophenocotton blue (Rawlins, 1933) was placed on the style and allowed to stain the tissues for a few minutes to an hour or two. The style was then crushed under a cover slip and examined. It might be necessary to remove the cover slip after crushing and restain, for the fluids of the style tissues seemed to exclude the stain solution for a time. These slides darkened gradually as more of the tissue took the stain. Epidermal and cortical cells of the style stained more slowly and to a lesser degree than the central strand of the style, which unfortunately stained as deeply as do the pollen tubes. These cells although joined in long strands, could not be mistaken for pollen tubes for they are twice the diameter of pollen tubes (6 micra against 3 micra) and, because they are plasmolyzed, they show cross-walls. Pollen tubes stained dark blue for some distance back of the tip, but did not stain over their entire length if they were long. Slides so prepared lasted for several weeks.

Another satisfactory stain was a saturated aqueous solution of Resorcin Blue (Lacmoid, C. I. No. 908) which was filtered and used immediately on styles which had been crushed or teased open or split in the manner already described. Like cotton blue, it acts as a cytoplasmic stain, but it also stains differentially the callose plugs of the pollen tubes. It was thus possible to see pollen tubes more or less throughout their length. The life of slides prepared in this manner was quite short.

No obvious or consistent difference was found in percentage of pollen which germinates, regardless of the compatibility of the pollination. In some cases a considerable amount of pollen failed to germinate, but this failure was not correlated with any particular type of cross (see table 9). Nearly all pollen tubes which failed were found to be fairly short. In incompatible pollinations of *L. peruvianum* exceptional tubes grew to a length of nearly

10 mm, but the great majority of incompatible tubes were less than 4.0 mm. These tubes were characterized by a swollen end, such as that described by Sears (1937) for *Petunia*.

Since swollen ends of pollen tubes have been found in every style examined regardless of the compatibility of the cross and even self-pollinations on the variety Pearson, they were not necessarily diagnostic of self-incompatibility. They were most distinct (all tubes appearing to have burst) and the tubes were shortest in the pollinations of *L. esculentum* on *L. peruvianum*.

If there was a "zone of inhibition" in the material in these experiments,

TABLE 9

TABULATION OF POLLEN TUBE BEHAVIOR IN VARIOUS CROSSES

Cross	Description	Pollen germination (per cent)
<i>per.</i> * bud check	no pollination, 4 grains only found, no germination.....	0
<i>per.</i> check	no pollination, 2 grains only.....	0
<i>esc.</i> self	style length: 7.4 mm, tube length max. 4.9 mm, end swollen; most tubes shorter....	50
<i>esc.</i> self	style length: 6.8 mm, tube 6.2 mm, end not seen; few tubes visible.....	45
<i>esc.</i> × <i>per.</i>	tube length: 0.5 mm, few tubes.....	25
<i>esc.</i> × <i>per.</i>	tube lengths: 5-8 mm, ends not swollen; few tubes short, ends swollen.....	80
F ₁ × <i>esc.</i>	tube lengths: 0.12, 1.2, 2.0 mm (max.) all ends swollen.....	45
F ₁ × <i>esc.</i>	tube lengths: 0.1, 0.025, 0.04 mm; most tube ends swollen.....	35
<i>per.</i> × <i>esc.</i>	tube lengths: 0.06, 0.15 mm; most ends swollen.....	10
<i>per.</i> × <i>esc.</i>	tube length: 0.4 mm; all ends swollen.....	40
<i>per.</i> × F ₁	tube length: 0.11, 1.0 mm most are 1-4 mm, ends slightly swollen.....	33
<i>per.</i> × F ₁	tube length: 0.4 mm, end swollen; 0.3 mm, end normal; most are 0.3-.5 mm, ends slightly swollen.....	75
<i>per.</i> self	style length 12 mm, tubes: 5.8 mm (max.) all ends swollen, most 2-4 mm long....	80
<i>per.</i> × <i>per.</i>	style length: 13 mm, tubes: 10.0 mm (max.) few swollen ends, most tubes 5-8 mm	75

* All styles were examined 18 hours after pollination. *per.* is *L. peruvianum*; *esc.* is *L. esculentum*; F₁ is hybrid, *esculentum* × *peruvianum*, no. 4-36.

it was immediately under the stigma, for it was here that most of the swollen ends were found in all styles examined. Rarely, however, has a swollen end been observed near the base of an incompatibly pollinated style in *L. peruvianum* or in selfed *L. esculentum*. The fact that a few tubes may grow very long even in incompatible tissue probably accounted for the occurrence of pseudo-fertility. No record of the vigor of this *L. peruvianum* plant was made.

A tabulation of the appearance of pollen tubes in a number of crosses is presented in table 9. Not all possible crosses are reported here, but the sample is representative. Although the appearance of swollen ends does not assure that the cross is incompatible, compatible and incompatible crosses differ visibly in their proportion of swollen ends.

Another visible difference between compatible and incompatible pollinations was revealed by aqueous lacmoid solution, which stains the callose plugs in the pollen tubes distinctly, especially if the material is well teased apart. Incompatible pollen tubes do not always grow long enough to form a callose plug. If one is formed, it is usually found just under the stigma. In compatible combinations examined 32 hours after pollination, however, these

plugs were found throughout the length of the style. In such cases, the pollen tube was empty of cytoplasm; therefore staining of the tube was limited to the growing tip ahead of the last plug.

Excised pollen tubes were examined; these showed the distance from the tip to the last callose plug to be between 0.3 mm and 0.4 mm in normal pollen tubes. This might indicate the distance a pollen tube must penetrate into the ovary before style removal fails to prevent fertilization. Also, since callose plugs are spaced fairly evenly in the pollen tube at a distance of .25 mm following the last plug, if all the plugs were counted in a cross-section of style .25 mm thick, the number of pollen tubes at that level of the style might be estimated fairly accurately.

Although genetic evidence suggests that two separate factors are responsible for inhibition of pollen in the style, cytological examination revealed no apparent difference in behavior of the pollen tubes to either factor except that the tubes inhibited by *S* factors may grow longer and be less swollen at their tips.

The swelling and bursting of incompatible pollen tubes seem to be more important factors in the incompatibility reaction in *Lycopersicon* than is any possible difference in rate of growth of compatible or incompatible tubes. The pronounced swelling of pollen tube ends might serve to distinguish this "active" incompatibility from the "passive" type found in *Brassica*, where different rates of growth account for success of compatible pollen and failure of incompatible pollen. This is corroborated by failure of incompatible crosses on flowers prevented from abscission by treatment with a "plant hormone," as noted on page 113.

SUMMARY

All tested plants of 11 accessions of the typical form of *Lycopersicon peruvianum* and 11 of its variety *dentatum* were found to be self-incompatible. Reactions tested extensively in one collection conformed exactly to the *Nicotiana* scheme. In addition, the other available species of the green-fruited subgenus *Eriopersicon*—*L. peruvianum* var. *humifusum*, *L. glandulosum*, and *L. hirsutum*—are self-incompatible, and the *Nicotiana* scheme seems to apply also to these. Correlated observations and limited mating tests of plants of the aforementioned species growing in their native habitat all indicated that the self-incompatibility is as rigid there as in cultures in California. The species of the red-fruited subgenus, *Eulycopersicon*, are self-fertile, as is also *L. hirsutum* f. *glabratum*.

Although the barrier to incompatible pollinations in *L. peruvianum* is very severe, a few seeds may be produced from incompatible matings by plants of slightly reduced vigor. All attempts to induce pseudo-fertility by means of the following methods failed: 1) various patterns of pruning; 2) treatment with a growth-promoting substance; 3) bud pollination; 4) compatible matings of other flowers on the same inflorescence; and 5) other means.

Hybrids of *L. esculentum* and *L. peruvianum* are as highly self-incompatible as the latter parent, but matings between hybrids of different parentage are highly compatible. As pistillate parents, these hybrids cross readily

with *L. peruvianum* but not with *L. esculentum*. As staminate parents, the hybrids mate successfully with *L. esculentum* but not with *L. peruvianum*. With a few exceptions the same mating relations were found between the F_2 and the parental species.

These compatibilities can be explained if it is assumed that the *S* oppositional factors act in the F_1 and F_2 hybrids in the same fashion as in *L. peruvianum*, but also oppose pollen bearing s_e , a recessive fertility allele from *L. esculentum*.

Reactions between F_1 and F_2 are less precise and agree with the hypothesis in the respect that nearly all crosses succeeded between the F_2 as staminate parents and the staminate F_1 , but do not conform in other combinations. Some of these deviations can be explained if it is assumed that two genes from *L. esculentum* that are independent of the *S* locus and of each other sporophytically render pollen incapable of fertilizing *L. peruvianum* whenever one or both are present.

Examination of styles after incompatible matings showed that the pollen germinates, but that the tubes usually grow only a short distance below the stigma, the end of the tube appearing swollen or burst.

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